REMARKS

After entry of this amendment, claims 42-47, 49, 50, 56-63, and 66-89 will be pending in this application. Claim 42 has been amended to recite a method of reconstituting an animal embryo, comprising transferring a donor nucleus from a diploid somatic cell into a first recipient oocyte. Support for diploid somatic cells is found throughout the specification, for example on page 11, .line 9. Claim 48 was canceled, and claims 49 and 50 have been amended to depend from claim 42 rather than claim 48. Claim 56 has been amended to recite a donor nucleus donated by a diploid cell. Support for diploid somatic cells is found throughout the specification, for example on page 11, line 9. Claims 69, 72, and 74 have been amended to correct the spelling of the word "fetus". Claims 77, 78 and 79 have been canceled. Claims 80-82 have been amended to recite cell lines or cell population obtained from ungulate embryos. Support for ungulates can be found throughout the specification, for example, on page 3, lines 27-29. New claim 89 is presented directed to a method of constructing a cell using the steps of claim 42.

The present invention provides an improved method for the production of an animal, animal embryo or reconstructed cell by *somatic cell* nuclear transfer.

The technology of using *somatic cell* nuclear transfer to produce animals was not developed until the mid-1990s. In 1995, Keith Campbell and Ian Wilmut produced two live lambs by nuclear transfer from cells from early embryos that had been cultured for several months in the laboratory. In July of 1996, Dolly the sheep was born. Dolly was the first mammal cloned from a cell from an adult animal. She was derived from cells that had been taken from the udder of a 6-year old Finn Dorset ewe and cultured in the laboratory. Each individual cell was then fused with an unfertilised egg from which the genetic material had been removed. Two hundred and seventy seven of these 'reconstructed eggs' - each now with a diploid nucleus from the adult animal were cultured for 6 days in temporary recipients. Twenty nine of the eggs that appeared to have developed normally to the blastocyst stage were implanted into 13 surrogate Scottish Blackface ewes. One gave rise to a live lamb, Dolly, some 148 days later. (see attached release from the Roslin Institute, "Nuclear transfer: a brief history", see also, Campbell et al. (1996) Nature 380, 64-66 and Wilmut et al. (1997) Nature 385, 810-813.). These

fundamental discoveries served as the basis for two patent application filings, which published as PCT applications, WO 97/07669 and WO 97/07668. These applications, which name Drs. Keith Campbell (also the present inventor) and Ian Wilmut as inventors, are currently pending throughout the world, and have resulted in the issuance of United States patents, including U.S. Patent Nos. 6,147,276, 6,252,133 and 6,525,243.

Although the technique developed by Campbell and Wilmut in 1995-1996 allowed for the cloning of animals, the technology was not highly efficient, and improved techniques to clone animals were desired. The present invention, discovered by Dr. Keith Campbell in 1999, provides an improved process to produce offspring through a nuclear transfer process. The new process advances the art by transferring the nucleus from the first recipient oocyte directly into a fertilized zygote or a preactivated second recipient oocyte, instead of allowing the first recipient oocyte to develop into an embryo first before transferring the nucleus to a second recipient cell. The first results of this improved technique, five cloned piglets, were published in Nature in 2000 (Polejaeva et al. Nature. 2000 Sep 7;407(6800):86-90). Prior to this discovery, nuclear transfer in pigs had not been very successful. A single piglet was reported after transfer of a blastomere nucleus from a four-cell embryo to an enucleated oocyte; however, no live offspring were obtained in studies using somatic cells such as diploid or mitotic fetal fibroblasts as nuclear donors (see, Prather et al. 1989 Biol. Reprod. 41: 414-418).

The present invention thus provides methods of reconstituting animal embryos or constructing a cell by (i) transferring a donor nucleus from a diploid somatic cell into a first recipient oocyte; (ii) removing the nucleus from the first recipient oocyte; (iii) either activating a second recipient oocyte or enucleating a fertilized zygote; and (iv) transferring the nucleus from the first recipient oocyte into the preactivated second recipient oocyte or the enucleated fertilized zygote.

Election/Restriction

The Applicant thanks the Examiner for withdrawing the election of species requirement.

Claim Objections

Claim 42 was objected to because it encompassed the use of a cell with any ploidy, despite the requirement that the claim be restricted to diploid cells. In response to this objection, the claims have been limited to the use of a diploid cell.

Claim Rejections: 35 USC § 101

The Examiner has rejected claims 77-82 under 35 U.S.C. 101 on the basis that the claims read on humans and animals. Claims 77-79 have been canceled. Claims 80-82 have been amended to recite isolated ungulate cell lines and cell populations.

Claim Rejections: 35 USC § 102

US Patent No. 5,843,780

The Examiner has rejected claims 77 and 80-82 under 35 U.S.C. 102(b) as anticipated by Thomson (US Patent No. 5,843,780). Claim 77 has been cancelled. Original claims 80-82 encompassed cell populations obtained from an embryo made by the method of claim 42. The Examiner asserts that the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes.

The '780 patent describes purified primate embryonic stem cells, as well as differentiated and undifferentiated cells derived from the primate embryonic stem cells. The Applicant has amended claims 80-82 to recite isolated ungulate embryonic stem cell lines or cell populations, undifferentiated ungulate cell lines or cell populations and differentiated ungulate cell lines or cell populations obtained from ungulate embryos produced by the method of claim 42.

Claim rejections: 35 USC § 102 and Double Patenting over US Patent No. 6,525,243

The Examiner has rejected prior pending claims 42-50 and 56-88 under 35 U.S.C. 102(e) and 35 U.S.C. 102(f) over Campbell *et al.* (US Patent No. 6,525,243), as well as under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims l-l6 of Campbell *et al.* (U.S. Patent No. 6,525,243).

As discussed above, the claimed process is different from the Campbell '243 patent in that the claims are limited to transferring the nucleus from the first recipient oocyte directly into a fertilized zygote or a preactivated second recipient oocyte. In contrast, the Campbell '243 patent describes allowing the first recipient oocyte to develop into a multicellular embryo first before transferring the nucleus to a second recipient cell. Campbell, in column 7, described that:

> Alternatively or additionally, it may be possible for increased yields of viable embryos to be achieved by means of the present invention by clonal expansion of donors and/or if use is made of the process of serial (nuclear) transfer. A limitation in the presently achieved rate of blastocyst formation may be due to the fact that a majority of the embryos do not "reprogram" (although an acceptable number do). If this is the case, then the rate may be enhanced as follows. Each embryo that does develop itself can be used as a nuclear donor at the 32-64 cell stage, alternatively, inner cell mass cells can be used at the blastocyst stage. If these embryos do indeed reflect those which have reprogrammed gene expression and those nuclei are in fact reprogrammed (as seems likely), then each developing embryo may be multiplied in this way by the efficiency of the nuclear transfer process. The degree of enhancement likely to be achieved depends upon the cell type. In sheep, it is readily possible to obtain 55% blastocyst stage embryos by transfer of a single blastomere from a 16 cell embryo to a preactivated "Universal Recipient" oocyte.

Due to this difference, the '243 patent does not anticipate the present claims.

With regard to the double patenting rejection, Applicant notes that none of the claims of the '243 patent include the elements of two nuclear transfer processes, nor do the claims refer to

the essential aspect of the present invention, which is transferring the nucleus from a first recipient oocyte directly into a fertilized zygote or a preactivated second recipient oocyte. The fact that the claims of the '243 patent could possibly dominate the practice of the present invention is not dispositive; the '243 claims must render the present claims obvious to maintain an obviousness-type double patenting rejection. Since the present claims are not described in nor suggested by the '243 patent or its claims, Applicant requests that the Examiner withdraw the double patenting rejection.

Claim Rejections -35 USC § 103

The Examiner has also rejected claims 42-50 and 56-88 under 35 U.S.C. 103(a) as unpatentable over Campbell *et al.* (WO 97/07669), Gurdon (J Cell Sci 4 :287-3 18, 1986) and Stice *et al.* (Biol of Reprod, 48 :715-719,1993). The Applicant notes that the Examiner makes no subsequent mention of the Stice reference in his basis for the rejection and that, rather, the Examiner references a page 232 of a Prather reference. The Applicant cannot respond to the Examiner's rejection over Prather without a copy of the reference or a sufficient citation of it. Given that the Applicant is not able to fully respond to this rejection without the Prather citation and is unclear on whether Stice is actually being asserted against the claims and if so, on what grounds, the next Office Action, if any, should be made non-final. The Applicant responds below to the rejections over Gurdon and Campbell.

WO 97/07669 provides the same general disclosure of serial nuclear transfer as the '243 patent, thus the same comments provided above regarding the '243 patent apply to this rejection. It took Dr. Campbell himself (one of the leading experts in the field) several years to develop a successful double nuclear transfer technique, subsequent to his earlier work. His improvement was heralded in *Nature,* which has among the highest standards for publication of scientific advancements. Clearly the community of his peers considered his work nonobvious and a true advance in the industry.

The Examiner suggests that Gurdon teaches that the reprogramming affect of the oocyte of the transferred nucleus is due to factors in the cytoplasm which reprogram the transferred nucleus rapidly. Gurdon's 1986 review was nine years before the first stunning disclosure by

Campbell and Wilmut of somatic cell nuclear transfer and ten years before the birth of Dolly. Gurdon only referred to techniques of embryonic nuclear transfer. Neither Gurdon nor Campbell suggested the method of transferring the nucleus from the first recipient oocyte directly into a fertilized zygote or a preactivated second recipient oocyte, instead of allowing the first recipient oocyte to develop into an embryo first before transferring the nucleus to a second recipient cell. There is no suggestion by either that the nucleus would be fully and adequately reprogrammed when removed at the oocyte stage, before the oocyte was allowed to mature to an embryo, much less that this procedure can result in more overall live births than somatic cell single nuclear transfer.

Conclusion

It is believed that this response places the application in condition for allowance. The undersigned counsel invites the Examiner to conduct a telephone conference to discuss any remaining issues.

Respectfully submitted,

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CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this Amendment and Response to Office Action, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450.

Stephanie D. Adams

October 14, 2004

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Nuclear transfer: a brief history

Dolly was the first mammal cloned from a cell from an adult animal. She was derived from cells that had been taken from the udder of a 6-year old Finn Dorset ewe and cultured in the laboratory. Individual cells were then fused with unfertilised eggs from which the genetic material had been removed. Two hundred and seventy seven of these 'reconstructed eggs' - each now with a diploid nucleus from the adult animal were cultured for 6 days in temporary recipients. Twenty nine of the eggs that appeared to have developed normally to the blastocyst stage were implanted into 13 surrogate Scottish Blackface ewes. One gave rise to a live lamb, Dolly, some 148 days later.

Cloning by nuclear transfer is not itself novel. The technique was first reported in frogs in 1952 and has been used widely since in amphibians to study early development (see **McKinnell, 1985** for a very readable review). This work showed that the first few cell divisions after fertilisation produce cells that are totipotent (i.e. they can develop into all of the cell types that make up the whole animal). As the embryo develops further, the cells lose this property and the success of nuclear transfer rapidly declines. Some nuclear transfer experiments using cells from adult frogs produced viable embryos, but these never developed beyond the tadpole stage.

Nuclear transler in mammals proved to be more difficult. The cloning of mice using nuclei from very early embryos was reported in 1977, but this work was not repeatable and interest among developmental biologists waned. Research on nuclear transfer in cattle continued, stimulated by the prospect of large commercial benefits from multiplying elite embryos. Artificial insemination allows each bull to have thousands of offspring but each cow can only produce 5 or 6 calves in a lifetime. Multiple ovulation embryo transfer (MOET) and cloning by embryo splitting have been used to partially redress this imbalance, but these techniques have limited potential for further development. By contrast, nuclear transfer has the ability, at least in principle, to produce an unlimited number of identical animals.

By the middle of the 1980's several research groups from around the world had produced cloned sheep and cattle by transferring nuclei directly from early embryos. Steen Willasden, working for Granada Genetics in the US, had produced live calves by nuclear transfer from embryos that had progressed to the 64- and 128-cell stage and this was the first suggestion that nuclear transfer in mammals was possible from at least partially differentiated cells.

In the early 1980's the then Animal Breeding Research Organisation had started research aimed at producing transgenic sheep and cattle that would secrete human proteins in their milk. Using the beta-lactoglobulin promoter, Dr John Clark and colleagues were able to direct expression of human genes specifically to the mammary gland. This success led to the setting up in 1987 of PPL Therapeutics and their subsequent production of Tracy, a transgenic sheep that secreted 35g of a human protein (alpha-1-antitrypsin) in each litre of her milk. Over the same period several other groups had also developed transgenic livestock and genetically modified pigs were beginning to be considered as sources of organs for transplantation to human patients.

At this time the only way of producing transgenic livestock was by pronuclear injection. This procedure involves the introduction of 200-300 copies of the transgene into a recently fertilised egg which is then implanted in a surrogate mother. Only 2-3% of eggs injected give rise to transgenic offspring and only some of these express the added gene at sufficiently high levels to be of commercial interest. It is also only possible to add genes by pro-nuclear injection.

If animals can be derived from cells in culture, then it is possible to carry out much more specific genetic modifications, including the removal or substitution of specific genes. This has been achieved in mice using embryonic stem (ES) cells, but to date no one has yet succeeded in obtaining ES cells from cattle, sheep or pigs. After learning of Willasden's success, Ian Wilmut thought that nuclear transfer might provide an alternative.

The major breakthrough came in 1995 when Keith Campbell, Ian Wilmut and colleagues produced live lambs - Megan and Morag - by nuclear transfer from cells from early embryos that had been cultured for several months in the laboratory (**Campbell et al., 1996**). The key element in this success was the induction of quiescence in the donor cells. However, at this stage they did not know if they had also stumbled on a particular amenable cell type simply by chance.

Additional experiments were performed to test if successful nuclear transfer was restricted to embryo-derived cells or could be carried out with a wider range of cell types. Nuclear transfer was carried out from embryo-derived cells, foetal fibroblasts and - in collaboration with PPL Therapeutics - cells from an adult ewe. Four lambs were born from embryo cells, three from the foetal cells and one, subsequently named Dolly, from an adult cell. Their identity was confirmed by DNA testing and the results published in Nature on 27 February 1997 (**Wilmut et al., 1997**).

For developmental biologists, Dolly's existence challenges one of the fundamental tenets of developmental biology. Most scientists had thought that differentiation - the gradual process of specialisation that allows the fertilised egg to develop into the hundreds of cell types that make up the whole animal- was irreversible. After all, even over a 90 year lifespan a liver remains a liver, a nerve cell a nerve cell. The production of a live lamb from a cell taken from the udder of a 6-year old ewe demonstrated that differentiated cells are not immutable. We do not know, however, the identity of the cell type that donated its nucleus to produce Dolly and it is possible that she derived from a mammary stem cell rather than a terminally differentiated epithelial cell.

Although it was Dolly that attracted the media attention, the key practical discovery in this sequence of events was made in 1995 with the production of live lambs from an established cell line. The Roslin Institute has applied for patents to cover its invention: these cover use in most of the countries of the world and in all animals.

The technology has now been taken one stage further. In July 1997, Roslin Institute and PPL Therapeutics announced the production of Polly, the first transgenic lamb produced by nuclear transfer. In this case, the donor cell was a foetal fibroblast that had been transfected with the gene coding for human blood clotting factor IX (**Schneike et al., 1997**). In the next 2-3 years, we hope to be able to perform more sophisticated genetic modifications in sheep and extend the technology to pigs for applications in xenotransplantation.

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